

Section 2: Identification of Cocaine and Crack

I. Introduction:

Cocaine and Crack samples will be analyzed by a microcrystalline test and run on GC/FID followed by confirmation on GC/MS. The microcrystalline test utilizes TLTA (di-p-toluoyl-l-tartaric acid) as a reagent. For cocaine samples, TLTA alone will produce crystallization. However, for crack samples, TLTA and Acetic Acid is used to produce crystallization. The purpose of the microcrystalline test is to determine the presence of the l-cocaine diastereoisomer.

II. Reagents:

- A.) TLTA (di-p-toluoyl-l-tartaric acid): 10 mg TLTA, 1mL Ethanol, 1mL glycerine in 8mL distilled water.
- B.) Acetic Acid (HOAC)
- C.) 9:1 Methylene Chloride/Isopropanol, Ethanol, or Methanol.
- D.) Methanol (solvent rinse for GC)
- E.) 0.1 N HCL: add 8.3mL concentrated HCL to sufficient water to make 1 L. (quantitation).
- F.) Cocaine HCL in C₂₄H₅₀ (quantitation standard).
- G.) 10% K₂HPO₄: dissolve 10g K₂HPO₄ in sufficient water to make 100mL. (quantitation).
- H.) C₂₄H₅₀ in Methylene Chloride (quantitation internal standard).
- I.) Anhydrous Sodium Sulfate (Na₂SO₄).

III. Equipment:

- A.) Microscope and slides
- B.) Analytical balance
- C.) Weigh paper
- D.) Pipettes
- E.) 50 mL volumetric flask
- F.) 25 mL volumetric flask
- G.) Stoppered test tubes (2)
- H.) 2 mL autosampler vials with Teflon caps
- I.) GC/FID: HP 6890 or 7890A
- J.) GC/MS: HP 7890A/5975C or HP 6890/5973 series.

IV. Procedure:

A.) Chromatography by GC/FID and GC/MS.

1. Obtain gross weight of sample evidence bag.
2. Remove one sample packet from the evidence bag and weigh.
3. Record individual packet gross weight in logbook.
4. Remove substance from packet onto a weigh paper.
5. Reweigh the empty packet and record the weight.
6. Add about 5 mg of sample to a labeled 2 mL autosampler vial.
7. Return remaining substance back to packet and reseal
8. Return packet(s) to original evidence back, reseal, and then put evidence bag in a new plastic bag and seal.
9. Subtract empty packet weight from total weight to obtain the substance's net weight.
10. For up to 100 packets, analyze 10% of the total. Report the statistical average of the individual net weights.
11. For more than 100 packets, analyze the square root of the total. Report the statistical average of all the individual net weights.
12. Add 1-2 mL of Ethanol, Methanol or 9:1 Methylene Chloride/Isopropanol to the vial and cap.
13. Place vial(s) on the GC/FID autosampler and run with the following sequence: Standard, Blank, Samples.
GC/FID conditions are as follows:

Method: EXP.M

Oven:

Initial Temp: 245°C

Initial Time: 0.00 min.

Rate: 10°/min.

Final Temp: 290°C
Run Time: 10 min.
Max. Temp: 325°C
Equilibration Time: 0.5 min.
Inlet:
Mode: split (35:1)
Initial Temp: 250°C
Pressure: 24.99 psi
Gas Type: Helium
Column:
Capillary: HP-1 30m x 320um
Initial Flow: 3.3 mL/min.
Detector:
Temp: 300°C
Hydrogen Flow: 30.0 mL/min.
Air Flow: 400 mL/min.
Makeup Gas: Helium

14. Obtain chromatographs. If cocaine is present, the instrument will detect a peak with a retention time around 3.40 minutes and will generate a report with accompanying chromatograph.
15. Check concentration to determine if dilutions are needed or if the injection volume needs to be increased for subsequent GC/MS run. Also observe any erroneous data that indicates that the sample may have to be reinjected.
16. Place same sequence on the GC/MS autosampler and run.
17. GC/MS conditions are as follows:
Method: EXP.M
Oven:
Initial Temp: 230°C
Initial Time: 0.00 min.
Max. Temp: 325°C
Equilibration Time: 0.50 min.
Rate: 10°/min.
Final Temp: 280°C
Run Time: 10 min.
Inlet:
Mode: split (50:1)
Initial Temp: 250°C
Pressure: 31.65 psi
Gas Type: Helium
Column:
Capillary: HP-1MS 25m x 200um x 0.33um
Max. Temp: 300°C
Initial Flow: 1.0 mL/min.

18. If cocaine is present in sample, the instrument will detect a total ion peak at approximate retention time of 3.50 minutes and will generate a report along with accompanying chromatograph and spectra. The spectra will contain the identity of the peak, its ion abundance and the ratio of ions 152:150, confirming the presence or absence of cocaine and the l-cocaine diastereoisomer. (see graph, last page)

B.) Microcrystalline Test:

1. On a microscope slide add a small amount of sample.
2. Add one drop of TLTA reagent to sample.
3. Crystallization should occur within a few minutes if the l-cocaine diastereoisomer is present.
4. If the sample does not crystallize within a few minutes, add one drop of HOAC to the sample on the slide. Observe under microscope at same magnification. Crystallization should occur within a few minutes, indicating that the sample is the free-base form of cocaine (crack).

C.) Quantitation Procedure:

1. Extract sample by weighing out 100 mg of sample in a 50 mL volumetric flask and bring to volume with 0.1 N HCL.
2. Record exact weight and calculate sample amount by dividing the weight by the final volume.
3. Prepare cocaine standard by weighing out 25 mg of Cocaine HCL in a 25 mL volumetric flask and bringing to volume with $C_{24}H_{50}$ in Methylene chloride.
4. In first stoppered test tube, labeled standard, add 2 mL of standard solution (prepared in step#3), 2 mL of 0.1 N HCL, and 1 mL of K_2HPO_4 .
5. In second stoppered test tube, labeled with sample number, add 2 mL of sample (prepared in step#1), 2 mL of 0.1 N HCL, and 1 mL of K_2HPO_4 . Two layers will form in each tube.

6. In labeled autosampler vials, add enough sodium sulfate (Na_2SO_4) to cover the bottom of the vial.
7. From each of the test tubes, pipette the bottom layer into the appropriate vial and cap.
8. Run the quantitation on the GC, with the following sequence: Cocaine Standard (inj.#1), Cocaine Standard (inj.#2, Calibration), Cocaine Standard (inj.#3), Blank, Sample(s), Cocaine Standard (inj.#4).
9. Check the standard to make sure recovery is at 100%. If not, rerun the standard (possibly at a higher injection amount).

V. Results:

- A.) For the microcrystalline test, record in logbook as TLTA positive, TLTA positive with HOAC, TLTA negative, or TLTA negative with HOAC.
- B.) Record results of the GC/MS in logbook. Then transfer the results to Drug Lab Result sheet that came with the actual samples. Be sure to include date of analysis, results, the number of packets analyzed, and signature.
- C.) All reports generated from the instruments should be filed so that they may be accessed at a later date, if necessary.

VI. Discussion:

When analyzing for cocaine, one must take into consideration the four diastereomers of cocaine. The four diastereomers are cocaine, pseudococaine, allococaine, and pseudoallococaine. Diastereomers are completely different chemical entities. All their measurable physical characteristics are different. These differences are measured by a number of different analytical methods. The one we use here is a microcrystalline test along with a GC/MS ion ratio. The ion ratios are different for each diastereomer. For cocaine, the ratio is between 1 and 2, for pseudococaine its 5 to 7, for allococaine its 7to10, and for pseudoallococaine its 3 to 5. The cocaine diastereomer is what is important to look for. It has two mirror image forms called l-cocaine and d-cocaine. The l-cocaine diastereomer is the form considered illegal. So for legal purposes, the sample must be analyzed for the presence of the l-cocaine

diastereomer. First, the microcrystalline test is used. The l-cocaine is confirmed when crystallization occurs with the addition of TLTA to the sample. If the sample is the free-base form of cocaine, acetic acid must be added along with TLTA to produce crystallization. Secondly, analysis by GC/MS will confirm the presence of l-cocaine by calculating the ion ratio of ions 152/150. If the result is less than two, the sample is positive for the presence of l-cocaine and is therefore illegal to possess.